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# CAP

(COMPLIANCE AUDIT PROGRAM)

10679

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**MILES** 

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September 2, 1992

Document Processing Center (TS-790)  
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Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460  
Attention: Section 8(e) Coordinator  
(CAP Agreement)



8EHQ-92-10679  
INIT 09/18/92



88920008968

RE: 8E-CAP-0065

Dear Sir:

Miles Inc. is submitting a study under the TSCA Section 8(e) Compliance Audit Program (CAP Agreement 8E-CAP-0065).

The tested chemical in the attached document is Polymeric MDI and can be represented by the CAS Registry Number 9016-87-9.

The title of the accompanying document is Acute Inhalation Toxicity Study of Polymeric MDI in Rats. This study is being sent in conjunction with the August 3, 1992 (8e CAP 0065) submission entitled: Acute Inhalation Toxicity of Diisocyanates, Polymer Isocyanates and Coating Systems on Rats by G. Kimmerle dated June 1976.

The results in this study that may be considered reportable according to the TSCA 8(e) Reporting Guide include acute inhalation toxicity data in the highly or extremely toxic range.

This compound has been the subject of a previous TSCA Section 8(d) submission OPTS 86-870000687.

The information submitted in this report is not considered Confidential Business Information.

If you have any questions on this submission, please contact me.

Sincerely,

*Donald W. Lamb*

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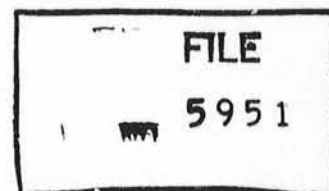


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Report no. V 82.050/212478

ACUTE INHALATION TOXICITY STUDY OF  
POLYMERIC MDI IN RATS  
(DRAFT)



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Project number:

B 81/2478

Start of the study:

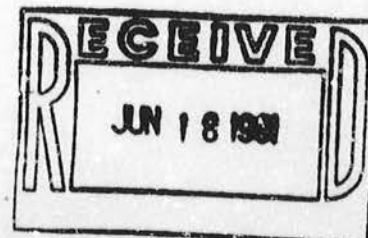
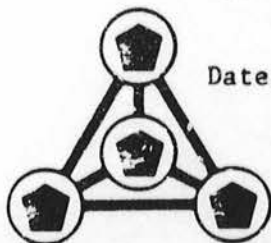
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19 May, 1981

Date:

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ABBREVIATIONS

MDI                   = 4,4'-diphenylmethane-di-isocyanate  
nitroreagent, NR   = N-(4-nitrobenzyl)-N-n-propyl-amine  
MDI-NR               = urea derivative of MDI and nitroreagent  
HPLC                 = high performance liquid chromatography



SUMMARY

1. The acute inhalation toxicity of polymeric MDI was studied by exposing groups of male and female rats one single time for 4 hours to different concentrations of an aerosol of this compound in the atmosphere.
2. For each concentration tested 10 males and 10 females were used: five males and five females for the LC<sub>50</sub> determination and the other animals for information on the gross pathology immediately after exposure.
3. Gross examination of animals killed immediately after the 4-hour exposure period revealed some haemorrhages or edema in the lungs. Similar changes were found in animals killed at the end of the observation period.
4. The 4-hour LC<sub>50</sub> of polymeric MDI in rats was found to be 490 mg/m<sup>3</sup> air.

ACUTE INHALATION TOXICITY STUDY OF POLYMERIC MDI IN RATS  
(DRAFT)

1. PURPOSE OF THE STUDY

It was requested by the International Isocyanate Institute Inc., U.S.A., to carry out an acute inhalation toxicity study with polymeric MDI in rats. Groups of male and female rats were to be exposed one single time for a period of four hours to an atmosphere in which a specific concentration of polymeric MDI aerosol was maintained, in order to determine the 4-hour  $LC_{50}$ -value of the test substance in rats. This value was calculated from the mortality percentages according to the probit analysis method of Finney (1).

2. MATERIALS AND METHODS

2.1 Test material

Samples of polymeric MDI (Desmodur 44 V 20) were received from Bayer AG, Leverkusen, FRG, on April 7, 1981 and April 14, 1981. Desmodur 44 V 20 is a very viscous ( $\eta = 200 \pm 40$  mpas), dark brown liquid with the following composition as specified by Bayer AG:

NCO - content	$30 \pm 2$ % (w/w)
hydrolysable chlorine	$\leq 0.3$ % "
total chlorine	$\leq 0.8$ % "
chlorobenzenes	$\leq 0.015$ % "
phenylisocyanate	$\leq 0.005$ % "
content of monomeric MDI	$52 \pm 3$ % "
content of sediment	$\leq 0.01$ % "

Oil red (0.5 %; w/w) was added to Desmodur 44 V 20 to facilitate the spectrophotometric determination of the concentration of the test material in the exposure atmospheres.



## 2.2 Test animals

Fifty male and fifty female young, adult SPF-bred rats (Cpb: WU, Wistar random) were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands. The initial mean body weight of the males was 167 g and that of the females was 139 g. They received the Institute's stock diet for rats and bottled unfluoridated tap-water ad libitum.

## 2.3 Exposure chamber

A stainless steel exposure chamber, having a capacity of  $1.5 \text{ m}^3$  was used.

The front door of the exposure chamber is provided with a glass window for observation of the animals and with several holes for the collection of air samples from different locations in the chamber.

## 2.4 Generation of the test atmosphere

A polymeric MDI aerosol was generated from a stainless steel/glass air nebuliser. The nebuliser was thermostated at about  $45^\circ \text{C}$ . A cyclone was placed behind the air nebuliser to remove large particles.

Upon entering the exposure chamber, additional air was mixed with the aerosol resulting in a total air flow of  $30\text{-}60 \text{ m}^3/\text{hour}$ .

## 2.5 Analysis of the test atmospheres

During the exposure period samples were taken from the test atmospheres. The concentration of polymeric MDI in these samples was determined by:

- a) Calculation from the oil red concentration, determined by spectrophotometry after sampling of the test atmosphere by means of a cascade impactor. The first sample was taken half an hour after the start of the exposure and subsequent samples were taken at one hour intervals.

- b) calculation from the oil-red concentration determined by spectrophotometry after sampling of the test atmosphere by means of the device depicted in figure 1 of the addendum. The start of sampling and the sampling frequency were the same as those indicated under a).
- c) calculation from the monomeric MDI concentration determined by HPLC after sampling of the test atmosphere by means of the device depicted in figure 1 of the addendum. The first sample was taken 45 min after the start of the exposure and subsequent samples were taken at approx. one hour intervals.

The particle size distribution in the polymeric MDI aerosol was determined in samples taken from the atmosphere in the chamber by means of a cascade impactor.

The different methods of sampling and analysis have been described in the addendum to this report.

## 2.6 Conduct of the study

Groups of rats, each consisting of 10 males and 10 females were exposed to the test material one single time for a period of 4 hours. Each group was sub-divided into two subgroups of five males and five females. The test was carried out with five different test substance concentrations.

During exposure the animals were housed individually in wire mesh stainless steel cages, which were located at one level in the exposure chamber. Individual housing was used to minimize filtration of inspired air by the animals' fur. During the exposure the animals were deprived of water and food.

Immediately after the exposure the survivors of one subgroup of rats, which was designated beforehand, were killed, while the survivors of the second subgroup were returned to their living cages for an observation period of two weeks. The living cages were suspended in an open rack in an animal room. The temperature and relative humidity in the room were controlled at  $21 \pm 1$  °C and 50-60 % respectively.

During the exposure and the observation period mortality and all reactions to treatment were observed and recorded daily. Body weights were recorded at days 0, 1, 2, 4, 7 and 14.

The survivors, which were killed by exsanguination from the abdominal aorta under ether anaesthesia, and the animals found dead were autopsied and examined for gross pathological changes. Lungs with trachea and larynx and the nose were preserved in a 4 % neutral aqueous phosphate-buffered formaldehyde solution.

## RESULTS

### 2.1 Analytical results

The individual and mean data on the concentration of polymeric MDI, established by means of oil-red determination and of MDI determined by HPLC, are summarised in table 1. On analysis of the test atmospheres large differences were often found between the results obtained by cascade impactor/spectrophotometry, glass filter/spectrophotometry or HPLC. In all cases the results obtained by HPLC were lower than those obtained by the other two methods. An explanation for these differences cannot be given at the moment.

The results of the particle size distribution measurements are given in table 2, which shows that in all tests more than 95 % of the particles was smaller than 5  $\mu$ m.

### 3.2 Clinical signs

During the exposure period the animals were quiet, kept their eyes closed and showed laboured respiration and mouth breathing, especially at the highest two levels. No mortality occurred during exposure.

The fur of the back skin of the animals was slightly pink at the end of the exposure period.

### 3.3 Mortality

The animals that died, died within 2 days after termination of the exposure period (table 3).

### 3.4 Body weights

The mean body weights of the animals are presented in table 4.

Both males and females lost body weight during the first two to four days of the observation period. Around day 4, the animals gained body weight again.

### 3.5 Gross pathology

Gross examination revealed some haemorrhages in the lungs and a haemorrhagic fluid around the nares of animals killed immediately after the exposure to 523, 418 and 384 mg polymeric MDI/m<sup>3</sup> air. In addition most lungs of animals exposed to 523 mg/m<sup>3</sup> air showed slight edema. *check into*

A few animals, which died during or were killed at the end of the two week observation period showed some haemorrhages in the lungs. This finding was most frequently observed in animals exposed to 418 mg polymeric MDI/m<sup>3</sup> air.

### 3.6 LC<sub>50</sub>-value

The 4-hour LC<sub>50</sub>-value of polymeric MDI in rats, based on concentration values, calculated by spectrophotometrical determination of oil-red (see Addendum section 2.1.1), was found to be 490 mg/m<sup>3</sup> with 440 and 540 mg/m<sup>3</sup> as the 95 % confidence limits.

## 4. REFERENCE

- 1) Finney, D.J., Probit analysis (3rd ed) London, Cambridge University Press, 1971.

Table 1 - Concentration of polymeric MDI in test atmospheres

Treatment Group <sup>1)</sup>	polymeric MDI (mg/m <sup>3</sup> )		
	calculated from spectrophotometry		calculated from HPLC
	Sampled by cascade impactor	Sampled by G4-filter	Sampled by G4-filter
A	619	380 <sup>2)</sup>	278
	450	380 <sup>2)</sup>	278
	482	447 <sup>2)</sup>	288
	447		
Mean	500	402	281
B	479	558	470
	524	604	516
	547	593	526
	542	593	540
Mean	523	587	513
D	403	456	376
	410	490	388
	426	501	378
	433	524	420
Mean	418	493	391
C	362	301 <sup>2)</sup>	211
	374	287 <sup>2)</sup>	223
	396	451	- 3)
	405	324	240
Mean	384	341	225

continued



For calculation of the  $LC_{50}$ -value the results of group B were not used, because the mortality in group B (zero) and the exposure concentration of group B ( $167 \text{ mg/m}^3$ ) were completely out of range.

These samples were taken with a G4-filter combined with a wash bottle filled with nitroreagent in  $\text{CH}_2\text{Cl}_2$ . No MDI could be detected in the absorption solution in the wash bottle, therefore the other samples were taken with a G4-filter only.

Not measured by mistake.



Table 2 - Particle size distribution in polymeric MDI aerosols

Aerodynamic Diameter, D <sub>50</sub> (μm)	distribution (%)		
	Sample 1	Sample 2	Sample 3
0.47	1.5	1.5	1.9
0.7	5.1	6.4	5.0
1.1	17.1	11.7	20.0
1.7	33.4	39.4	39.9
2.5	28.0	26.1	23.9
3.4	10.4	10.0	6.2
4.3	4.2	4.5	2.9
5.7	0.4	0.3	0.3
7.7 and >		0.1	

Table 3 - Mean concentration of polymeric MDI in the test atmospheres and the corresponding mortality data

Treat ment group	Mean concentration of polymeric MDI <sup>1)</sup> (mg/m <sup>3</sup> )	Number of deaths at day								mortality (%)
		0 <sup>2)</sup>		1		2		3 - 14		
		♂	♀	♂	♀	♂	♀	♂	♀	
	500	-	3	2	1	-	-	-	-	60 4+2
	523	1	-	2	1	-	1	-	-	50 2+3
	418	-	1	1	-	-	2	-	-	40 3+1
	384	-	-	-	1	-	-	-	-	10 1.0

1) Mean values of samples taken by means of the cascade impactor and analysed by spectrophotometry.

2) Day 0 is day of exposure.

Table 4 - Mean body weights (g) of groups of male and female rats exposed to different concentrations of polymeric MDI.

Mean concentration of polymeric MDI (mg/m <sup>3</sup> )	Body weight at day (g)					
	0*)	1	2	4	7	14
MALES						
500	168	148	137	155	177	224
523	164	149	142	128	139	186
418	170	151	-1)	153	173	208
384	164	141	133	-1)	151	205
FEMALES						
500	136	127	118	112	130	153
523	138	124	115	118	137	164
418	143	129	-1)	129	149	170
384	139	120	116	-1)	138	158

\*) Day 0 is day of exposure; weights are recorded just prior to the exposure.

1) By mistake the males and females were not weighed on these days.

ADDENDUM TO REPORT NO V 82.050/212478  
ACUTE INHALATION TOXICITY STUDY  
OF POLYMERIC MDI IN RATS

Analytical methods for the determination  
of polymeric MDI in aerosols

Authors :                      Dr A.W.J. de Jong  
                                    Drs L.M. Appelman

Approved by :                  Dr P. Slump

Date :                          June, 1982

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#### A. MATERIALS

1. Dry toluene: Toluene, p.a (Merck 8325, Darmstadt, F.R.G.) was dried over KOH-pellets for 1 hour. After filtration, it was further dried and kept over sodium wires.
2. Dry dichloromethane: Dichloromethane, p.a (Baker 7053, Deventer, the Netherlands) was dried over anhydrous sodium sulphate.
3. Methanol, p.a. (Merck 6009, Darmstadt, F.R.G.).
4. Ethanol, absolute (Zuid-Nederlandse Spiritus, Bergen op Zoom, the Netherlands).
5. Hexane (Merck 4368, Darmstadt, F.R.G.).
6. HPLC solvent: Hexane (A.5)/ethanol (A.4) 4:1 (v/v).
7. HPLC column: Spherisorb S5W (Phase Separations, Queensferry-Clwyd, U.K.) in a 250 x 4.6 mm id stainless steel tubing (filled by Chrompack, Middelburg, the Netherlands).
8. 0.05 % nitroreagent in dry toluene or dry dichloromethane. Nitroreagent as the hydrochloride salt was delivered by Bayer AG (Leverkusen, F.R.G.). The 0.05 % solutions were made according to Keller et al. (Ref. 1).
9. MDI-NR derivate as reference material was delivered by Bayer AG (Leverkusen, F.R.G.).

#### B. APPARATUS

1. Air Flow measurement. An anemometer (type 641 N, W. Lambrecht KG, Göttingen, F.R.G.) was used for air flow measurements.
2. Air sampling:
  - a. Air samples for analysis by HPLC were collected with the device as depicted in figure 1.
  - b. Air samples for spectrophotometric analysis were collected with an eleven stage cascade impactor (as described by A. Bürkholz (Ref. 2)) or with the sampling device depicted in figure 1.



3. Spectrophotometric determinations. A Pye Unicam SP 8400 spectrophotometer was used for the spectrophotometric determinations.
4. HPLC determinations. The chromatographic system consisted of:
  - a. A Varian LC 5020 pumping system, a Varian Autosampler LC 8055 and a Valco AH-60-0000K sampling valve with a 10  $\mu$ l sample loop.
  - b. A Spherisorb S5 column (A.7).
  - c. A Pye Unicam LC 47-3 detector, a Varian CDS 111 data system and a Kipp A41 recorder.
5. Particle size determinations. For particle size determinations an eleven stage cascade impactor and a microbalance were used.

#### C. ANALYTICAL PROCEDURES

##### 1. Sampling of the test atmospheres

Air samples were taken with the cascade impactor (B.2.b) or with the sampling device depicted in figure 1, using an air flow at the rate of 5 l/min. Both air samplers were installed through an opening in the front door in such a way, that the air samples were taken at the level in the exposure chamber where the animals were placed.

The cascade impactor was used for obtaining samples for the determination of the particle size distribution and of the total mass concentration of polymeric MDI aerosol in the test atmosphere. In the latter case the residues on the eleven discs of the impactor were taken up into dry dichloromethane (A.2) and diluted to 25 ml in a measuring flask. This solution was analyzed spectrophotometrically.

The sampling device of figure 1, with or without wash bottle, was used for the determination of the mass concentration of polymeric MDI by spectrophotometric analysis. When used, the wash bottle was filled with 25 ml absorption solution (A.8), consisting of 0.05 % nitroreagent in dry dichloromethane. In case the wash bottle was used, the G4 glass filter was washed with the absorption solution followed by 10 ml dry dichloromethane (A.2) after the air sampling. In other cases the glass filter was washed with dichloromethane only.

For the determination of monomeric MDI by HPLC analysis the sampling device was used with wash bottle and absorption solution. After the air sampling, the glass filter was washed with the absorption solution followed by 10 ml dichloromethane. The wash solutions were quantitatively brought into a 50 ml measuring flask and dichloromethane was added to the mark.

## 2. Analysis of the test atmospheres

### 2.1 Determination of polymeric MDI concentration

#### 2.1.1 Spectrophotometric analysis

The concentration of polymeric MDI in the test atmosphere was determined indirectly by spectrophotometric analysis of the final air sampling solution obtained as described in section C.1.

The spectrophotometric analysis was based on the presence of the dyestuff oil red in the polymeric MDI. The absorbance of the final air sampling solution was determined at 530 nm with respect to dichloromethane as the reference. Also, a calibration curve was constructed for the absorbance E versus the concentration of oil red in dichloromethane solutions. The concentration of polymeric MDI in the test atmosphere was calculated according to the formula:

$$\text{polymeric MDI in the test atmosphere} = \frac{ExV \times 200 \times 1000}{gV \times F \times l} \text{ mg/m}^3$$

in which: E = absorbance at 530 nm  
V = volume of final air sampling solution (ml)  
F = calculation factor obtained from the calibration curve  
(ml/mg. cm)  
l = optical path-length (cm)  
gV = volume of air sampled (l)  
200 = conversion factor for obtaining the corresponding  
concentration of polymeric MDI

### 2.1.2 HPLC-analysis

The concentration of polymeric MDI in the test atmosphere is determined indirectly by HPLC quantification of MDI-NR derivate in the final air sampling solution obtained according to section C.1, using the sampling device depicted in figure 1.

This quantification is based on comparison of the sample chromatogram with a chromatogram obtained by injection of an equal volume of a standard solution of derivatized MDI (A.9) in dichloromethane (external standard). The HPLC analysis was carried out by means of straight phase liquid chromatography. The method is a modification of the method of Dunlap et al. (Ref. 3). The chromatographic system outlined in B.4 was used.

#### Analytical procedure:

Prior to use, the silica column is washed with methanol, dichloromethane and hexane successively (c. 30 ml of each). Then the column is equilibrated to the HPLC solvent: c. 20 % ethanol in n-hexane (A.6), degassed by ultrasonification. The rate of solvent flow is set at 2.0 ml/min and the column effluent is monitored at 254 nm and 0.16 AUFS. After pumping through c. 30 ml, the equilibrium is checked by two successive injections of the external standard solution. Equal retention times must result.

A 10  $\mu$ l aliquot of the final air sampling solution (C.1) is used for quantification by HPLC (in duplicate). For calibration (external standard), a 35 ppm solution of derivatized MDI (A.9) in dry dichloromethane (A.2) was used. This represents 0.35  $\mu$ g MDI-NR derivate. The minimum detectable quantity of MDI-NR derivate is c. 1.5 ng at 0.04 AUFS with the above described HPLC system.

After use, the column is washed with 100-150 ml of n-hexane and stored. The concentration of MDI-NR derivate in the sample solution is calculated by comparison of peak areas obtained with sample and calibration solutions, using a peak integrating chromatography data system (B.4). The concentration of monomeric MDI in the test atmosphere is calculated from the MDI-NR derivate concentration in the sample solution taking into account the volume of air that has been sampled.

The concentration of polymeric MDI in the test atmosphere is calculated from the monomeric MDI concentration using the formula:

$$\text{polymeric MDI in test atmosphere} = \frac{A_s \cdot V_s \cdot M_c}{A_c \cdot V_i \cdot V_{\text{air}}} \times 1.92 \text{ mg/m}^3$$

in which:

$A_s$	= area of MDI-NR in sample chromatogram
$A_c$	= area of MDI-NR in calibration chromatogram
$V_s$	= volume of final air sampling solution (ml)
$V_i$	= injection volume (ml)
$V_{\text{air}}$	= volume of air taken from test atmosphere ( $\text{m}^3$ )
$M_c$	= mass of MDI-NR in calibration chromatogram (mg)
1.92	= conversion factor because polymeric MDI contains 52 % monomeric MDI

## 2.2 Determination of the particle size distribution of the aerosol

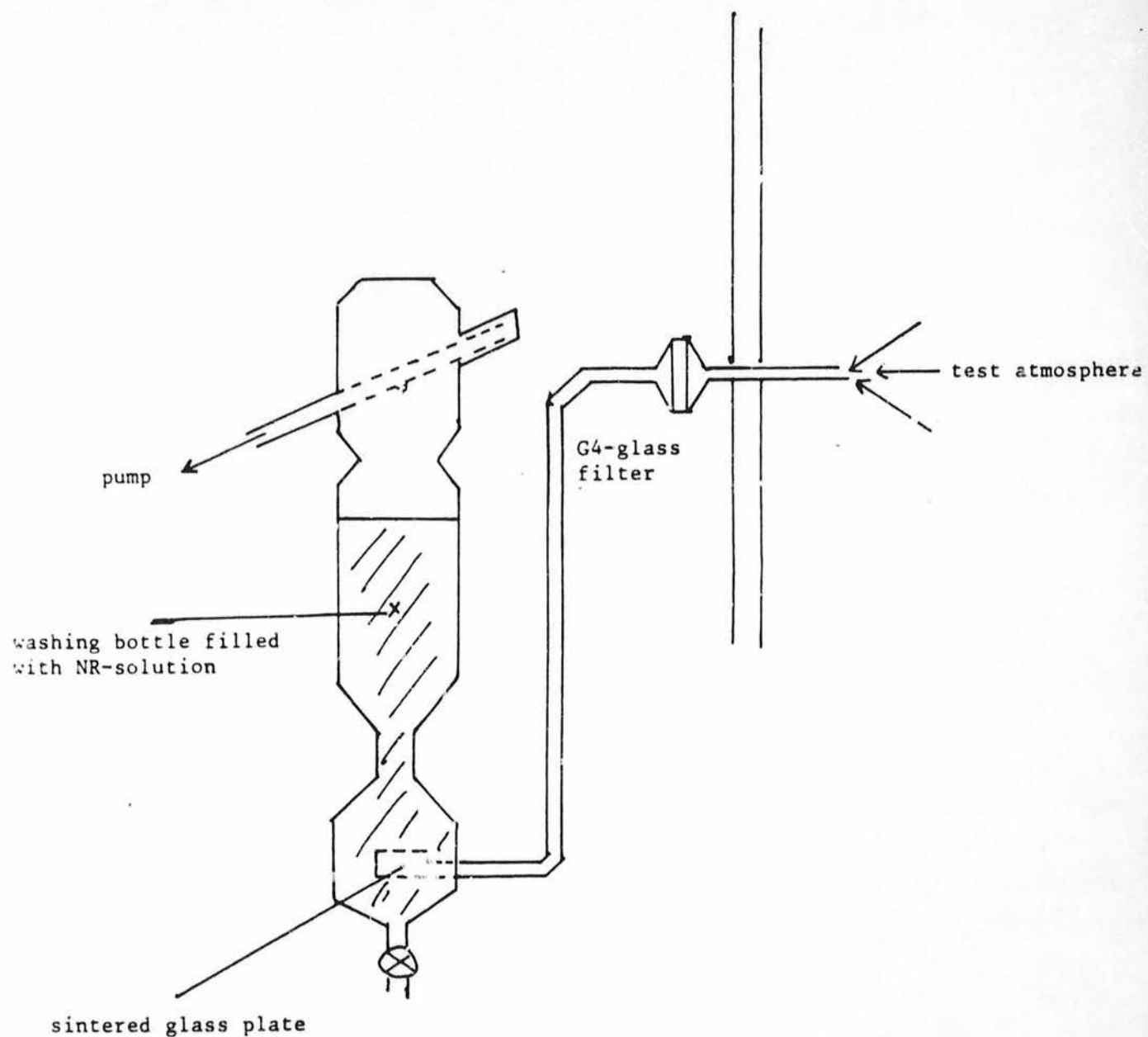
The particle size distribution was determined by weighing the discs of the cascade impactor (B.5) after air sampling as described in (C.3). The weighing was carried out with a micro-balance (8.5).

The weight of each particle size fraction was then expressed as a percentage of the total mass of polymeric MDI in the test atmosphere sample.

## D. REFERENCES

1. Determination of isocyanates in the working atmosphere by thin layer chromatography. J. Keller, K.L. Dunlap and R.L. Sandridge. Anal. Chem. 46 (1974) 1845 (the solvent benzene should and can be replaced by toluene or dichloromethane).
2. Eichuntersuchungen an einem Kaskadenimpaktor. A. Birkholz. Staub-Reinhaltung Luft 33 (1973) no 10, 397.
3. Determination of isocyanates in working atmospheres by high speed liquid chromatography. K.L. Dunlap, R.L. Sandridge and J. Keller. Anal. Chem. 48 (1976) 497.

Figure 1. Air sampling device.





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